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CDP-Choline: Neuroprotection in Transient Forebrain Ischemia of Gerbils

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CDP-choline is a rate-limiting intermediate in the biosynthesis of phosphatidylcholine (PtdCho), an important component of the neural cell membrane. The ability of CDP-choline to alter phospholipid metabolism is an important function in the treatment of ischemic injury. Exogenous treatment with CDP-choline stimulates PtdCho synthesis and prevents release of free fatty acids (FFA), especially arachidonic acid (AA), after ischemia/reperfusion. Phase III clinical trials of CDP-choline in the treatment of stroke are currently underway. Here we report the neuroprotection by CDP-choline in transient forebrain ischemia of gerbils. CDP-choline significantly attenuated the blood-brain barrier (BBB) dysfunction after ischemia with 6-hr reperfusion, and considerably reduced the increase of AA in FFA and leukotriene C_4 (LTC_4) synthesis at 1 day. Edema was significantly elevated after 1 and 2 days, but attained maximum at 3-day reperfusion. CDP-choline substantially attenuated edema at 3 days. Ischemia resulted in 80 ± 8% CA_1 hippocampal neuronal death after 6-day reperfusion, and CDP-choline provided 65 ± 6% neuroprotection. CDP-choline may act by increasing PtdCho synthesis via two pathways: (1) conversion of 1,2-diacylglycerol to PtdCho, and (2) biosynthesis of S-adenosyl-L-methionine, thus stabilizing the membrane and reducing AA release and metabolism to leukotriene C_4. This would result in decreased toxicity due to AA, leukotrienes, oxygen radicals, lipid peroxidation, and altered glutamate uptake, thus limiting BBB dysfunction, edema and providing neuroprotection. J. Neurosci. Res. 58:697–705, 1999.

Key words: arachidonic acid; leukotriene C_4; CA_1 neuronal death; phosphatidylcholine; hippocampus; S-adenosyl-L-methionine; apoptosis

INTRODUCTION

CDP-choline is an essential intermediate in the Kennedy biosynthetic pathway of the membrane phospholipids and is a rate-limiting factor in the phosphatidylcholine (PtdCho) biosynthesis (D'Orlando and Sandage, 1995; Kennedy and Weiss, 1956; Schabitz et al., 1996; Secades and Frontera, 1995; Weiss, 1995). The ability of CDP-choline to alter phospholipid metabolism may be an important function in the treatment of ischemic injury (Aronowski et al., 1996; D'Orlando and Sandage, 1995; Murphy and Horrocks, 1993). When administered intraperitoneally (i.p.) or orally, CDP-choline is hydrolyzed to choline and cytidine. Once absorbed, cytidine and choline disperse widely throughout the organism, cross the blood-brain barrier (BBB), and are resynthesized into CDP-choline (Fig. 4). In cerebral ischemia, the accumulation of cytidine 5′-monophosphate resulting from ATP depletion increases the conversion of PtdCho to 1,2-diacylglycerol (DG) and free fatty acids (FFA) including arachidonic acid (AA). Administration of CDP-choline reduced the release of FFA, particularly AA, by stimulating the PtdCho synthesis, and showed an improvement in the neurological outcome (Lopez-Coviella et al., 1995; Murphy and Horrocks, 1993; Schabitz et al., 1996; Trovarelli et al., 1981). It has also been demonstrated that CDP-choline: (1) restored the ATPase activities and reduced cerebral edema (Secades and Frontera, 1995), and (2) decreased lipid peroxidation (Fresta et al., 1994; Kasner and Grotta, 1997). Pharmacological action of CDP-choline may extend beyond the effect on phospholipid metabolism, since its metabolites (such as cytidilic nucleotides, choline, methionine, betaine; Fig. 4) are involved in numerous metabolic pathways (Galletti et al., 1991). Choline deficiency is associated with: (1) decreased membrane PtdCho and sphingomyelin, (2) release of DG and ceramide, (3) activation of a caspase, and (4) induction of apoptosis (Blusztajn, 1998; Holmes-McNary et al., 1997; Yen et al., 1999).

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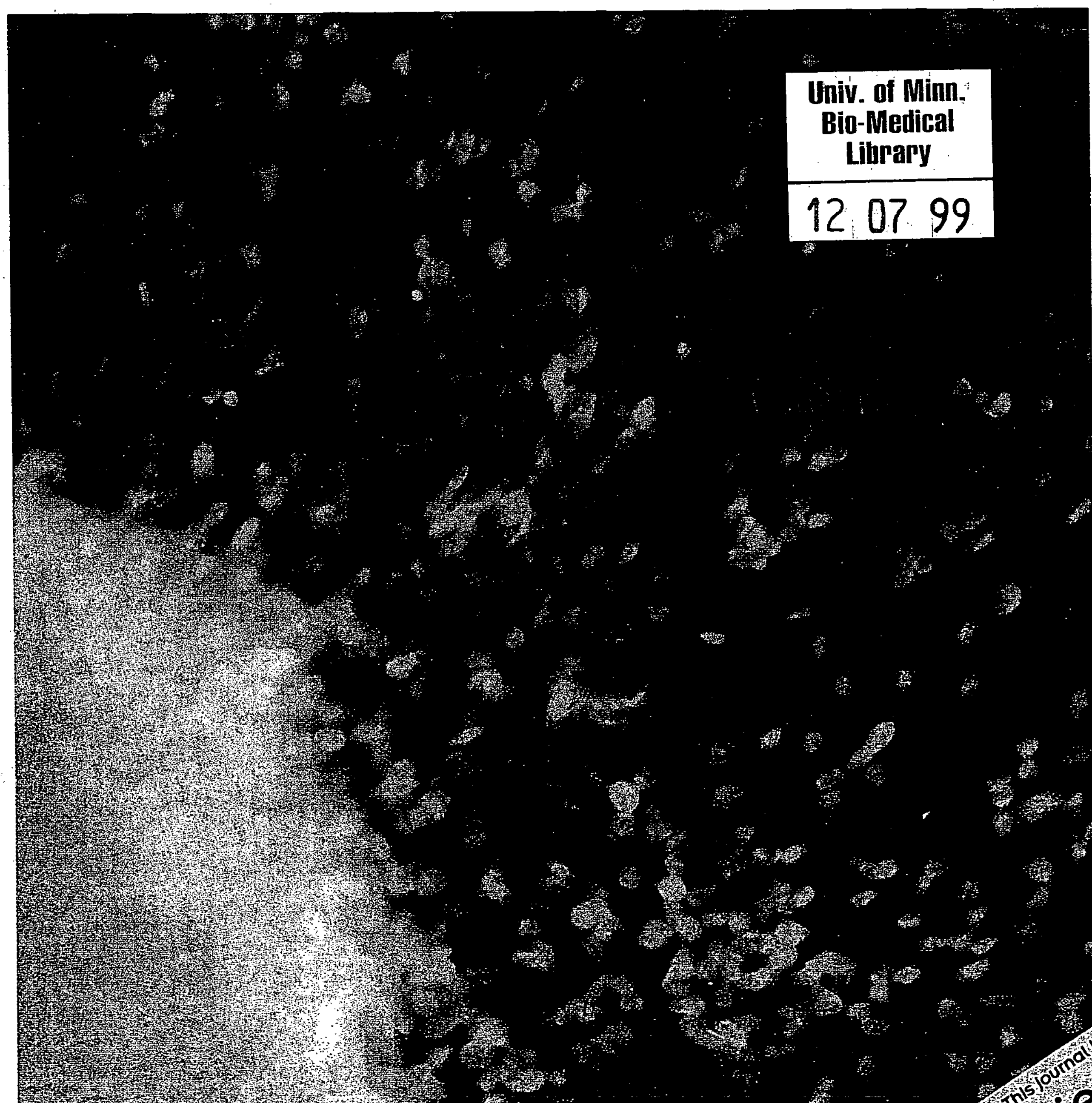

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CDP-choline has shown neuroprotective effects in cerebral ischemia, hypoxia, traumatic brain injury, Alzheimer's disease, Parkinson's disease, learning and memory disorders, and appears to reduce neurologic deficit in recent clinical trials with no serious side effects (Aronowski et al., 1996; Clark et al., 1997, 1998; Dixon et al., 1997; D'Orlando and Sandage, 1995; Onal et al., 1997; Schabitz et al., 1996, 1999; Secades and Frontera, 1995; Weiss, 1995). Phase III clinical trials of CDP-choline in the treatment of stroke are currently underway.

Earlier studies examined the effects of CDP-choline on phospholipid metabolism in central nervous system (CNS) injury (Arrigoni et al., 1987; Horrocks et al., 1981; Lopez-Coviella et al., 1995; Trovarelli et al., 1981) but did not correlate these biochemical changes with physiological outcome. In the present study, the effect of CDP-choline on biochemical changes (attenuation of AA release and conversion to leukotriene C_4 [LTC_4]) were correlated with the physiological outcome (BBB dysfunction, edema, and neuronal survival) in transient forebrain ischemia of gerbils. To the best of our knowledge, this is the first report showing protection of the CA_1 hippocampal neurons by CDP-choline in transient forebrain ischemia of gerbils.

MATERIALS AND METHODS

Materials

The following materials were obtained from the indicated suppliers: chemicals and lipid standards (Sigma Chemicals, St. Louis, MO), CDP-choline (BIOMOL, Plymouth Meeting, PA); high-performance liquid chromatography (HPLC) grade solvents (Fisher Scientific, Pittsburgh, PA), thin-layer chromatography (TLC) plates (Analtech, Newark, DE), BondElut C_{18} columns (Varian Associates, Harbor City, CA), and LTC_4 ELISA kits (Cayman Chemicals, Ann Arbor, MI).

Transient Forebrain Ischemia

All surgical procedures were conducted according to the animal welfare guidelines set forth in the NIH Guide for the Care and Use of Laboratory Animals (US Department of Health and Human Services Pub 85–23, 1985) and were approved by the animal care committee of the University of Wisconsin-Madison. Male Mongolian gerbils (50–80 g) were anesthetized with 1% halothane in 70:30 N_2O:O_2. Both carotid arteries were exposed (with the aid of a surgical microscope) by a horizontal neck incision, occluded with aneurysm clips for 10 min and then reperfused for up to 6 days (Rao et al., 1997, 1998a,b, 1999). Brain temperature was measured by means of a thermocouple probe placed in the temporalis muscle (Busto et al., 1989). Body and cranial temperatures were maintained at 37–38°C and 36–37,°C, respectively, using a thermostatically controlled water blanket and heating lamp. Physiological variables were monitored and regulated for the sham and ischemic groups during the anesthesia, and for 3-hr postischemia reperfusion. Brains of the anesthetized gerbils were frozen in situ, and cortices and hippocampi were dissected at 0°C for lipid analysis and LTC_4. Anesthetized gerbils were decapitated for BBB dysfunction, edema, and histopathology.

Dose and Administration of CDP-Choline

CDP-choline was administered to gerbils at 500 mg/kg i.p. This dose was virtually without side effects in recent studies (Aronowski et al., 1996; D'Orlando and Sandage, 1995; Lopez-Coviella et al., 1995; Schabitz et al., 1996). CDP-choline did not alter the physiological parameters (blood pressure, PaO_2, $PaCO_2$, pH, rectal and brain temperatures) compared to saline treated controls (Schabitz et al., 1996). For lipid analysis, LTC_4 measurements and BBB dysfunction, CDP-choline was given to gerbils just after the end of ischemia and at 3-hr reperfusion. For edema studies, gerbils were given CDP-choline just after ischemia, 1 and 2 days. CDP-choline was given to gerbils just after ischemia and thereafter every day up to 5 days for histopathology.

Measurement of DG and FFA (Including AA)

All solvents and extracts were purged with nitrogen during the extraction, TLC, and methylation of lipids. FFA and DG lipids from brain tissue were extracted into chloroform:methanol (1:2, v:v) containing 0.01% butylated hydroxytoluene (BHT) and 10 nmol of heptadecanoic acid (17:0) as internal standard for FFA determination (Bligh and Dyer, 1959; Dhillon et al., 1995; Rao et al., 1999). FFA and DG were separated on silica gel G TLC plates using petroleum ether:ether:acetic acid (80:20:1 v/v/v). The DG and FFA bands were identified using authentic standards (Sigma) and were scraped into 1 mL methanol containing 0.01% BHT. Blank regions of the TLC corresponding to DG and FFA were also analyzed to determine any TLC background contribution. Lipids were converted to methyl esters by adding 20 μL concentrated sulfuric acid and heating at 70°C for 30 min. The methyl esters were extracted into hexane and separated with a Hewlett Packard 6890 gas chromatograph (GC) using a capillary column (HP crosslinked FFAP) and equipped with an autosampler. Quantification was based on external standard calibration with 17:0 as internal standard. TLC blanks did not show any GC peaks corresponding to AA (20:4), palmitic (16:0), stearic (18:0), and oleic (18:1) acids.

LTC_4

$LTC_4$was quantified as described (Baskaya et al., 1996) with minor modifications (Rao et al., 1999) using Cayman Chemicals enzyme-linked immunosorbent assay (ELISA) kit. The frozen hippocampi and cortices were homogenized in 6 mL of methanol containing 0.1% acetic acid. After centrifugation at 4°C, 16,000 × g for 20 min, the supernatants were brought to 25% methanol by addition of 0.1% acetic acid, and loaded onto prewashed 2 mL BondElut C_{18} columns. After washing with 0.1% acetic acid and 0.1% acetic acid in 25% methanol, LTC_4 was eluted with 0.1% acetic acid in 90% methanol. The eluates were dried under N_2 and resuspended in ELISA buffer. Eighty-five percent of LTC_4 standard was recovered under these extraction conditions (Rao et al., 1999).

BBB Dysfunction

Anesthetized gerbils were given 2% Evans blue (25 mg/kg i.v.) 1 hr before sacrifice and were perfused with saline before decapitation. Hippocampi and cortices were dissected, homogenized in 50% trichloroacetic acid, and centrifuged at 16,000 g for 20 min. The supernatant was diluted with ethanol. Evans blue fluorescence (excitation 620 nm, emission 680 nm) was quantitated (Rao et al., 1997, 1999; Uyama et al., 1988).

Edema

Anesthetized gerbils were decapitated, the hippocampi were dissected and immediately weighed to yield wet weight. After drying in a desiccated oven for 2 days at 70°C, hippocampi were reweighed for dry weight. The percentage water in the tissue was calculated according to the formula: {(Wet weight − Dry weight)/Wet weight} × 100.

Histopathology

Gerbils were anesthetized 6-days after ischemia and perfused transcardially with perfusion wash and buffered paraformaldehyde as described (Kirino and Sano, 1984; Rao et al., 1997, 1999). Brains were removed, postfixed for a day, sectioned (10-μm-thick) coronally and were stained with thionine. The hippocampal CA_1 neurons/mm were counted as described (Kirino et al., 1986; Rao et al., 1997, 1999).

Statistical Analysis of Data

Data were presented as mean ± S.D., and analyzed using a one-factor analysis of variance (ANOVA) with the Bonferroni test to compare between the groups (GraphPad Software, San Diego, CA). A value of $P < 0.05$ was considered significant.

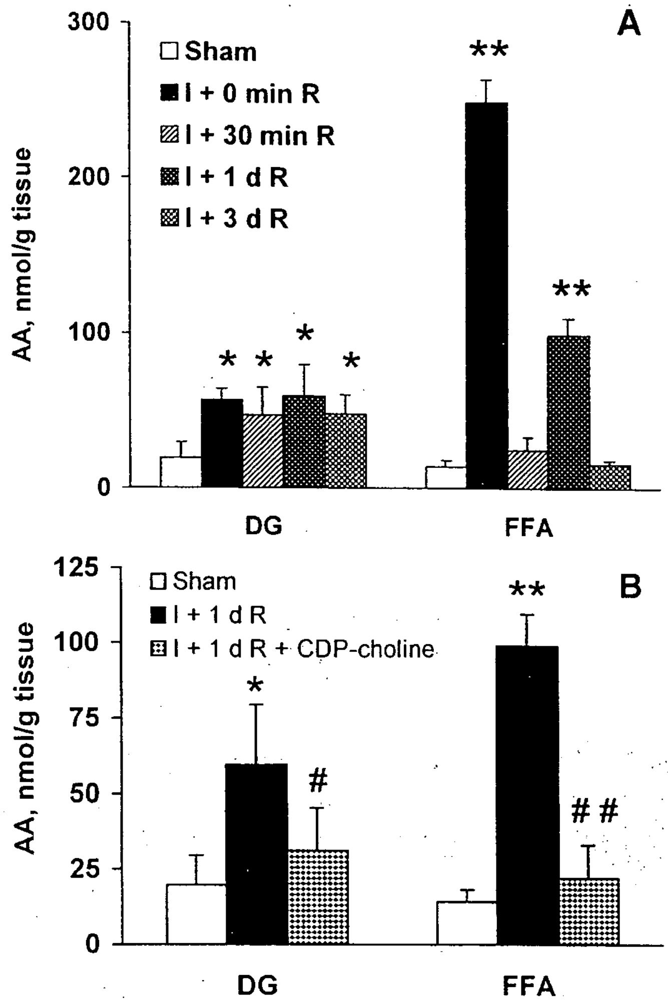


Fig. 1. **A:** Hippocampal arachidonic acid (AA) levels in free fatty acids (FFA) in sham and ischemic groups from 0 to 3 days. **B:** Effect of CDP-choline on AA levels in FFA after 10-min forebrain ischemia and 1-day reperfusion (R). Sham animals treated with CDP-choline showed no effect on AA levels. N = 7 per group; $*P < 0.05$, and $**P < 0.01$ compared to sham; $\#P < 0.05$ compared to ischemic group and not significant compared to sham; $\#\#P < 0.01$ compared to ischemic group and not significant compared to sham. DG, 1,2-diacylglycerol.

RESULTS

Cortices and hippocampi of shams treated with CDP-choline showed no effect on biochemical (DG, FFA, and LTC_4) or physiological (BBB dysfunction, edema and histopathology) parameters.

DG and FFA

A substantial increase of hippocampal AA ($P < 0.01$ compared to shams) in FFA occurred during 10-min ischemia which returned to sham levels after 30-min reperfusion (Fig. 1A). There was also a significant increase ($P < 0.05$ compared to shams) in the AA levels

in DG. These results are in agreement with the previous observations (Abe et al., 1987, 1989; Ikeda et al., 1986; Nakano et al., 1990; Rao et al., 1999). Similar results were observed for total fatty acid content of DG and FFA.

Treatment with CDP-choline prior to ischemia did not significantly alter the release of AA following 10-min ischemia with no reperfusion (data not shown). Since there was no significant effect of CDP-choline (i.p.) on lipid metabolism during ischemia, our subsequent studies focused on the effect of CDP-choline on metabolic events during reperfusion.

TABLE I. Effect of CDP-Choline on Blood-Brain Barrier Dysfunction After Transient Forebrain Ischemia†

	Evans blue, μg/g tissue	
	Cortex	Hippocampus
Sham	9.5 ± 0.6	9.8 ± 0.7
I + 6-hr R	42.9 ± 4.7*	61.6 ± 9.2*
I + 6-hr R + CDP-choline	23.2 ± 3.2**	29.6 ± 4.6**

†I, ischemia; R, reperfusion; Data are presented as mean ± S.D. (n = 7 per group).
*$P < 0.01$ compared to shams.
**$P < 0.01$ compared to shams and compared to ischemic.

CDP-Choline Decreased AA Levels in FFA

A later release of AA was observed after 1-day reperfusion ($P < 0.01$ compared to sham), which is also in agreement with the other studies (Abe et al., 1989; Nakano et al., 1990). CDP-choline attenuated AA content of DG ($P < 0.05$ compared to untreated ischemic) and FFA ($P < 0.01$ compared to untreated ischemic) at 1-day reperfusion (Fig. 1B).

CDP-Choline Reduced LTC_4 Levels

Our earlier studies showed an elevation in LTC_4 levels after transient ischemia (Baskaya et al., 1996; Rao et al., 1999). Measurement of hippocampal LTC_4 after 1-day reperfusion (n = 7 per group) showed that levels were significantly elevated (21.6 ± 2.3 ng/g tissue compared to sham 1.87 ± 0.4; $P < 0.01$; Rao et al., 1999), corresponding with release of AA (Fig. 1A). CDP-choline significantly reduced these levels (6.2 ± 1.5 ng/g tissue; $P < 0.01$ compared to untreated ischemic and $P < 0.05$ compared to sham). Similar changes were observed in cortices (data not shown).

CDP-Choline Attenuated BBB Dysfunction

A significant amount of Evans blue ($P < 0.01$ compared to shams; Table I) was extravasated into the ischemic hippocampus at 6-hr reperfusion (Rao et al., 1999). CDP-choline significantly attenuated ($P < 0.01$ compared to untreated ischemic group) the BBB dysfunction after transient ischemia, but did not completely restore the BBB integrity ($P < 0.01$ compared to shams; Table I). Similar changes were also observed in cortex. This observation is in agreement with other studies that CDP-choline reestablished the BBB integrity in rat ischemia models (Secades and Frontera, 1995).

CDP-Choline Reduced Edema

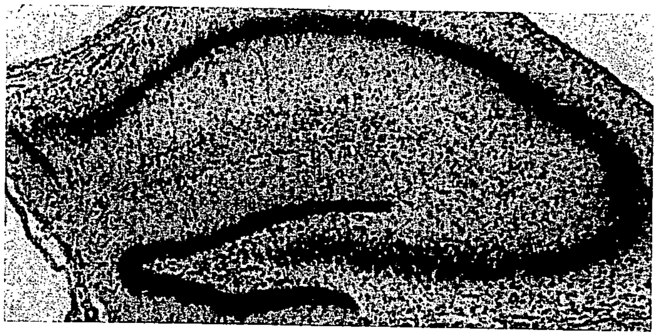
The time course of edema in transient ischemia of gerbils over 3-day reperfusion has been measured in the hippocampus (n = 7 gerbils per group). Significant edema (expressed as percentage water content) was observed after 1-day (80.46 ± 0.8; $P < 0.05$ compared to sham 78.74 ± 0.3) and 2-day (80.57 ± 1.04; $P < 0.05$ compared to sham), but maximum edema was attained at 3-day reperfusion (82.39 ± 1.65; $P < 0.01$ compared to sham). Our results also show that CDP-choline significantly attenuated edema after 3 days (79.48 ± 0.3; $P < 0.01$ compared to 3-day untreated ischemic; not significant compared to shams) in transient ischemia.

CDP-Choline Protected CA_1 Hippocampal Neurons

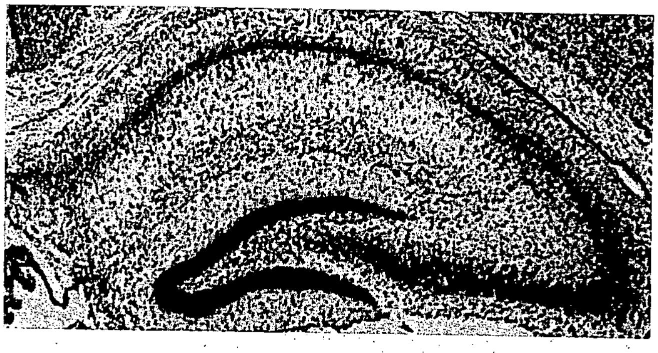
The vulnerability of the hippocampal CA_1 region to neuronal death after transient ischemia has been demonstrated (Kirino and Sano, 1984; Rao et al., 1997, 1999). Representative profiles of sham gerbils subjected to 10-min ischemia and 6-day reperfusion, and ischemic gerbils treated with CDP-choline are shown in Figure 2. Ischemia produced 80 ± 8% neuronal death in the CA_1 region compared to shams (Fig. 3; n = 7 per group; $P < 0.01$ ischemic vs. shams). CDP-choline protected 65 ± 6% of the CA_1 hippocampal neurons (Fig. 3; n = 7 per group; $P < 0.01$ compared to untreated ischemic; $P < 0.05$ compared to shams). Shams treated with CDP-choline showed no change in neuronal counts.

DISCUSSION

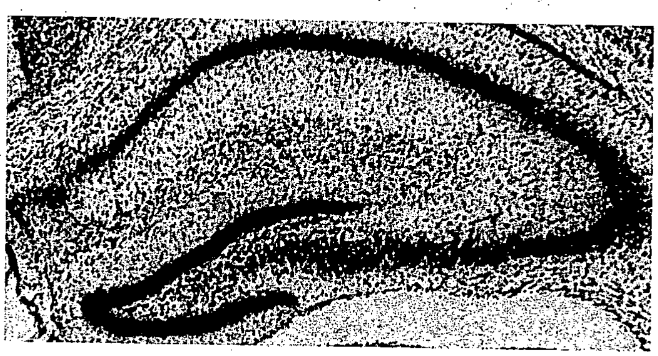
Cerebral ischemia and reperfusion initiate a complex series of metabolic events leading to neuronal death. The degradation of membrane lipids and accumulation of FFA, particularly AA, is one such critical event (Katsuki and Okuda, 1995). AA is released during ischemia by the activation of phospholipase C (PLC) and Phospholipase A_2 (PLA_2). PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate DG and inositol-1,4,5- trisphosphate (Rhee and Bae, 1997). DG is further converted by DG-lipases to FFA and AA and thus serves as an intermediate source of FFA. PLA_2 catalyzes the hydrolysis of AA at the *sn*-2 position of phospholipids such as PtdCho and phosphatidylethanolamine (PtdEtn) and is a major pathway contributing to AA (Bonventre et al.,



A. Sham



B. Isch. + 6 d reper.



C. Isch. + 6 d reper. + CDP-Choline

Fig. 2. CDP-choline. Neuroprotection in the hippocampal CA_1 region after forebrain ischemia and 6-day reperfusion. **A:** Sham. **B:** Ischemic. **C:** Ischemic + CDP-choline. N = 7 per group. Representative profiles are shown.

1997; Farooqui et al., 1997) accumulation in ischemia/reperfusion.

The accumulation of cytidine 5′-monophosphate resulting from ATP depletion increases the PtdCho hydrolysis to DG, which is further metabolized to FFA includ-

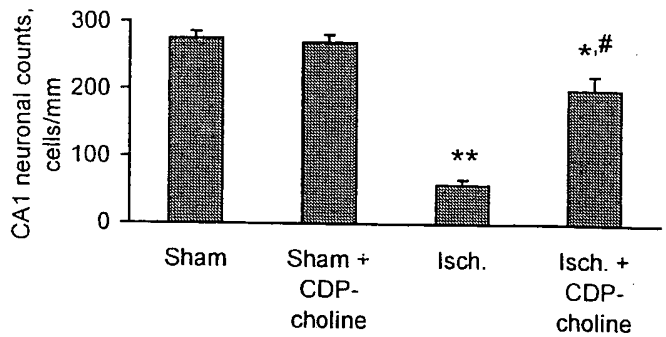


Fig. 3. Effect of CDP-choline on CA_1 hippocampal neuronal counts in the gerbil hippocampus following 10-min forebrain ischemia and reperfusion for 6-days (n = 7 per group). ** $P < 0.01$ compared to sham; *$P < 0.01$ compared to untreated ischemic; and #$P < 0.05$ compared to shams.

ing AA. Restoration of PtdCho synthesis may attenuate formation of DG and subsequent AA release (Fig. 4; D'Orlando and Sandage, 1995; Weiss, 1995).

AA, its lipoxygenase and cyclooxygenase metabolites, and free radicals formed during its metabolism, reduce glutamate uptake processes (Barbour et al., 1989). This may result in a large influx of calcium due to glutamatergic excitation, leading to sustained activation of both PLC and PLA_2 and later release of AA at 1-day reperfusion (Abe et al., 1989). The attenuation of AA could reduce this feedback activation of PLC and PLA_2 (Barbour et al., 1989; Katsuki and Okuda, 1995; Lombardi et al., 1996).

The lack of effect of CDP-choline on AA levels during ischemia (no reperfusion) in our studies may be due to lower brain concentrations when CDP-choline was given i.p. Intracerebroventricular administration of CDP-choline prior to onset of ischemia (which may have provided high concentrations in brain) prevented the release of AA during permanent ischemia of gerbils (Horrocks et al., 1981; Trovarelli et al., 1981). CDP-choline decreased AA release at 1 day to near sham levels (Fig. 1B). This is the first report showing that CDP-choline given i.p. reduced the AA levels after ischemia and 1-day reperfusion. This may have resulted by CDP-choline: (1) accelerating PtdCho biosynthesis from DG, and (2) stabilizing the membrane by preventing the phospholipid hydrolysis. The observed decrease in DG levels after treatment with CDP-choline (Fig. 1B) may partly account for the decrease in FFA. CDP-choline was also reported to inhibit PLA_2 activation (Arrigoni et al., 1987; Gimenez and Aguilar, 1998; Knapp and Wurtman, 1999; Mykita et al., 1986) and this aspect has not been completely explored. Administered CDP-choline is absorbed as its components, cytidine and choline. The pattern of metabolites in brain tissue indicates efficient salvage of choline into phospholipids and of the cytosine moiety into nucleic acids (Galletti et al., 1991).

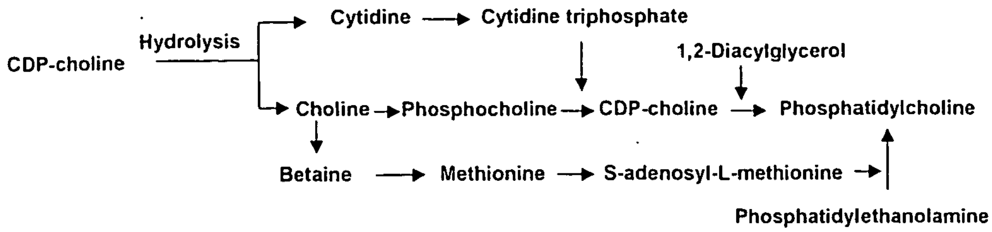


Fig. 4. CDP-choline metabolism and phosphatidylcholine biosynthesis.

LTC_4, a vasoactive metabolite of AA formed by the action of 5-lipoxygenase, has been implicated in BBB dysfunction and edema (Baba et al., 1991; Baskaya et al., 1996; Betz et al., 1989; Wahl et al., 1988) associated with ischemia (Rao et al., 1999). The decrease in AA by CDP-choline after 1-day reperfusion was reflected in a reduction in LTC_4 levels (see LTC_4 in Results section). Since CDP-choline does not have any known effect on 5-lipoxygenase (which converts AA to LTC_4), the decrease in LTC_4 levels are probably attributable to reduction in AA levels. Glutamate-dependent neurotoxicity also induces cyclooxygenase activity and cyclooxygenase-2 expression (Kaufmann et al., 1997; Ohtsuki et al., 1996). Our earlier studies also demonstrated an elevation in the cyclooxygenase products of AA (prostaglandins and thromboxanes) after transient forebrain ischemia (Dempsey et al., 1986). The decreased AA availability may have also attenuated their formation.

Loss of BBB integrity at 6-hr reperfusion was attenuated by CDP-choline (Table I). BBB permeability may promote neuronal death through extravasation of proteins and toxic metabolites from serum (Nishino et al., 1994; Preston et al., 1993). Since phospholipids are hydrolyzed by the activation of phospholipases in ischemia (Abe et al., 1989; Ikeda et al., 1986; Nakano et al., 1990), repair of cell membranes by resynthesis of PtdCho may be important. S-adenosyl-L-methionine (AdoMet), which attenuated the BBB dysfunction and CA_1 hippocampal neuronal death (Rao et al., 1997), can serve as the methyl donor in the biosynthesis of PtdCho from PtdEtn (Fig. 4; Sato et al., 1988). Choline liberated from CDP-choline can be converted to AdoMet via metabolism to methionine (Fig. 4; Cestaro, 1994; Galletti et al., 1991). Methionine is one of the major metabolites present in the brain after CDP-choline administration (Galletti et al., 1991). Thus, CDP-choline may increase PtdCho synthesis to stabilize the membrane via two pathways: (1) biosynthesis of AdoMet, and (2) conversion of DG to PtdCho (Kennedy and Weiss, 1956; Weiss, 1995). These pathways of CDP-choline metabolism are outlined in Figure 4.

Significant edema developed after 1- and 2-day reperfusion, but was maximum at 3 days compared to shams (Rao et al., 1999). CDP-choline decreased the edema at 3-day reperfusion. A number of factors including AA and its metabolites have been implicated in BBB dysfunction and edema after CNS insult (Betz et al., 1989; Katsuki and Okuda, 1995; Rao et al., 1999; Wahl et al., 1988). AA itself may intercalate into the membrane lipid layer, thus altering membrane structure and permeability. AA metabolites (leukotrienes, prostaglandins, and thromboxanes) have been linked to vasogenic edema (Chan and Fishman, 1984; Kaufmann et al., 1997). LTC_4 has been implicated in BBB dysfunction and edema associated with ischemia (Baba et al., 1991; Baskaya et al., 1996; Betz et al., 1989; Ohtsuki et al., 1995; Rao et al., 1999; Wahl et al., 1988). Oxygen radicals (Chan, 1996; Chan et al., 1998) formed during the metabolism of AA (Hall, 1996; Watanabe and Egawa, 1994; Werns and Lucchesi, 1990; Yamamoto et al., 1997) result in formation of lipid peroxides (Watanabe et al., 1994) and disruption of membrane function. Thus the release of AA after 1-day reperfusion may result in edema by production of lipid peroxides, LTC_4, prostaglandins, and thromboxanes. Down-regulation of γ-glutamyl-transpeptidase (which converts LTC_4 to LTD_4) and marked loss of activity at 3 days after ischemic brain injury (Baba et al., 1991) coincides with the maximum edema at 3 days. Thus, the decrease in edema by CDP-choline may have resulted by limiting AA, the generation of lipoxygenase/cyclooxygenase products, and lipid peroxides (Fresta et al., 1994). Furthermore, it has been reported that CDP-choline restored the ATPase activities and minimized edema in experimental models (Murphy and Horrocks, 1993; Secades and Frontera, 1995).

The pattern of the CA_1 hippocampal neuronal death in gerbils subjected to 10-min ischemia and 6-days reperfusion (Fig. 2B) was similar to that previously described (Kirino and Sano, 1984). Our results showed that CDP-choline provided neuroprotection in the CA_1 hippocampal region (Fig. 2C). This is in contrast to an earlier study wherein a lower dose of CDP-choline (76 mg/kg i.p. administered once) did not show any protection on CA_1 neurons in rat forebrain ischemia (Sato et al., 1988).

AdoMet also showed neuroprotection on the CA_1 region (Matsui et al., 1987; Rao et al., 1997; Sato et al., 1988) and may have operated through membrane stabilization mechanisms (Trovarelli et al., 1983). Since CDP-

choline can be metabolized to AdoMet (Fig. 4), this pathway may have contributed to the neuroprotection exerted by CDP-choline.

Our study showed that treatment with CDP-choline in transient forebrain ischemia attenuated the AA release, BBB dysfunction, edema, and subsequently protected the hippocampal CA_1 neurons. Recently it has been shown that production of superoxide anion radicals was significantly elevated in vulnerable CA_1 neurons after reperfusion injury (Chan et al., 1998; Yamaguchi et al., 1998). CDP-choline, by minimizing AA release, is likely to have decreased oxygen radical generation associated with oxidative metabolism of AA, which may have partly contributed to the observed neuroprotection. To our knowledge, this is the first report documenting the neuroprotective effects of CDP-choline on CA_1 hippocampal neurons in transient ischemia models. Possible mechanisms that are supported by other studies include neuronal membrane stabilization by PtdCho synthesis (Lopez-Coviella et al., 1995), reduction in FFA including AA (D'Orlando and Sandage, 1995; Horrocks et al., 1981; Schabitz et al., 1996; Trovarelli et al., 1981), free radical formation (Kasner and Grotta, 1997), lipid peroxidation (Fresta et al., 1994), and glutamate toxicity (Clark et al., 1998; Katsuki and Okuda, 1995; Lombardi et al., 1996).

CDP-choline provided only partial neuroprotection in our studies. Therapeutic efficiency of CDP-choline may be enhanced by: (1) combination with CDP-ethanolamine (Murphy and Horrocks, 1993; Secades and Frontera, 1995), (2) liposome encapsulation (Fresta and Puglisi, 1997; Fresta et al., 1994), or (3) synergistic combination with other neuroprotective agents. CDP-choline, in combination with the N-methyl-D-aspartic acid (NMDA) receptor antagonist MK-801 (Onal et al., 1997) or with basic fibroblast growth factor (Schabitz et al., 1999), provided enhanced neuroprotection in cerebral ischemia. Future therapeutic treatments may also need to take into account the combined activities of both arms (lipoxygenase and cyclooxygenases) of AA metabolism, since these two enzyme systems are linked by a common substrate.

The exact neuroprotective mechanisms of CDP-choline in the treatment of CNS injury need further investigations. Clinical trials of CDP-choline have initiated treatment up to 1 day after the onset of stroke (Clark et al., 1997; Kasner and Grotta, 1997). In our studies, CDP-choline treatment initiated immediately after the end of ischemia showed significant neuroprotection. Studies are in progress to determine whether beneficial effects will be obtained if the treatment is delayed after the onset of reperfusion, and such studies may have implications for the clinical use of CDP-choline.

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NOTE ADDED IN PROOF

Neuroprotection by CDP-choline may also involve increased sphingomyelin (SM) synthesis from the pro-apoptotic agent, ceramide (Hannun and Obeid, 1995; Perry and Hannun, 1998) via the pathway (CDP-choline + DG → PtdCho; PtdCho + ceramide → SM + DG).

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